USER MANUAL

Roti®-Prep Blood Genomic DNA MINI

For isolation of DNA from whole blood

Ord. No. 8620

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Introduction and product description:

- Preparation by well-known mini spin-column system
- Fast, easy and efficient
- Preparation in approx. 25 minutes
- Yield up to 30 µg gDNA

Roti®-Prep Blood Genomic DNA MINI has been specially designed for the isolation of high purity genomic DNA from blood samples.

The isolation is carried out through the proven and reliable spin column technique, which is very easy to handle and needs only few steps.

The Roti®-Prep Blood Genomic DNA MINI kit has been adapted to gain high purity DNA from fresh or frozen blood (EDTA or citrate stabilised) or whole blood, respectively. In order to get optimal results, two protocols, depending on sample amount, are provided. Furthermore, the properties of the binding buffer have been optimised to reach highest binding capacity with blood samples, permitting a yield of up to 30 µg gDNA out of 400 µl blood.

Utilisation is uncomplicated and fast to achieve (approx. 25 minutes, lysis included), and offers best conditions for following downstream applications.

Isolated gDNA is free of EDTA, RNA, or proteins, and may directly be applied to all standard downstream applications like, for instance, restriction digest, PCR etc.

For research use only.

The kit is intended for use by professional users and **does not provide a diagnostic result**. It is the sole responsibility of the user to use and validate the kit in conjunction with all required downstream in vitro assays.

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Cautions:

*Lysis Buffer LSN** **Warning** H319-H400 P273-P280-P305+P351+P338

Binding Buffer BR **Danger** H225-H319-H336
P210-P280-P303+P361+P353-P305+P351+P338-P312

Proteinase K **Danger** H315-H319-H334-H317-H335
P280-P284-P333+P313-P337+P313-P342+P311

Washing Solution WSO **Danger** H225-H319-H336
P210-P280-P303+P361+P353-P305+P351+P338-P312

*In case of forming of precipitates, please dissolve by careful warming.

MSDS: the appropriate MSDS can be downloaded from our website www.carlroth.com.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

Literature Citation: When describing a procedure for publication using this product, please refer to it as *Carl Roth's Roti®-Prep Blood Genomic DNA MINI Kit*.

1. Materials provided with the kit and storage conditions

Amount	Component	Storage
6 / 2x30 / 6x30 mg	Proteinase K	+4 °C / -20 °C*
12 / 25 / 120 ml	Lysis Buffer LSN	>20 °C**
8 / 50 / 250 ml	Binding Buffer BR	RT
8 / 30 / 120 ml	Washing Solution WSO (ready-to-use)	RT
2 / 10 / 2x18 ml	Washing Buffer WSH (Base)	RT
2x2 / 15 / 2x30 ml	Elution Buffer EB	RT
10 / 50 / 250	Mini spin columns (red)	RT
50/ 250 / 1.250	1.5 ml Elution tubes	RT
10 / 50 / 250	2 ml Collection tubes	RT

* Lyophilized Proteinase K should be stored at +4 °C. Prior to use, dissolve Proteinase K in 0.3 / 1.5 / 5x1.5 ml sterile, distilled water as given below. Dissolved Proteinase K should be stored in aliquots at -20 °C. Avoid repeated freeze&thaw cycles for each tube.

** Lysis Buffer should be stored at over 20 °C in order to prevent precipitation of reagents.

The Roti®-Prep Blood Genomic DNA MINI Kit should be stored dry, at room temperature (14-25 °C). Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming. This kit is stable for at least 6 months after receipt, when stored as directed.

Contents of this Kit may not be bought separately.

The components of each Roti®-Prep Blood Genomic DNA MINI Kit were tested by isolation of genomic DNA from whole blood samples in general, followed by spectro-photometrical measurement, gel electrophoresis and target-amplification. The user is responsible, however, to validate the performance of the Roti®-Prep Blood Genomic DNA MINI Kit for any particular use, since the performance characteristics of our kits have not been validated for specific applications.

2. Required Material and Equipment not included in this kit

- Ethanol 96-99.8 %, e.g. Ethanol p.a. (e.g. 9065.1)
- Distilled, sterilized H_2O
- RNAse A, stock solution 100 mg/ml (e.g. 7156.1, 100 mg solubilized in 1 ml distilled water, made DNAse-free by cooking)
- PBS sterile, diluted to 1x (e.g. from 1058.1, Roti®-Stock 10 x PBS)

3. Application

Roti®-Prep-Blood Genomic DNA MINI is designed for isolation of high-purity genomic DNA from whole blood. Spin column based preparation allows elution in a small volume of low-salt buffer, eliminating time-consuming phenol-chloroform extraction or alcohol precipitation. The columns may be used either in micro-centrifuges or on vacuum manifolds.

The kit allows the extraction of **up to 30 µg** gDNA per preparation from common blood collection systems up to 200 µl or up to 400 µl. If smaller volumes of blood are used, apply sterile PBS up to 200 µl final sample volume.

The kit co-purifies DNA and RNA, if both are present in the sample. In case RNA-free genomic DNA is required, RNase A has to be added to the sample after lysis.

The Roti®-Prep-Blood Genomic DNA MINI is not for use with cell-free body fluids such as cerebrospinal fluid, serum, plasma or urine, tissue or stool samples. The kit performance has not been evaluated with buffy coat, cultured or isolated cells, swabs, dried blood spots and viral DNA. The kit is also not specified for the isolation and purification of fungal, bacterial or parasite nucleic acids.

The kit is intended for use by professional users and **does not provide a diagnostic result**. It is the sole responsibility of the user to use and validate the kit in conjunction with all required downstream in vitro assays.

During lysis, the sample has to be mixed carefully and, if possible, constantly. Reduced movement of the lysis mix will reduce the lysis efficiency, and, subsequently, the recovery rate of gDNA. We recommend using a shaking platform or thermomixer in order to keep the sample at constant movement.

Repetition of elution (final step) often enhances the recovery rate overall. However, even so often, concentration of the recovered gDNA is reduced in parallel, since the whole amount of buffer used for elution is higher. Elution is 100 µl Elution Buffer EB, followed by another elution step in 100 µl Elution Buffer EB, may be recommended.

Store the extracted DNA at +4 °C. For long time storage placing at -20 °C is recommended.

For centrifugation we recommend a standard microcentrifuge. Centrifugation steps should be carried out at room temperature.

Avoid *repeated* freeze&thaw cycles for the blood to be extracted.

Before start, be sure to

- Add Ethanol (96-99.8 %, not incl. in this kit) to the Washing Buffer WSH as follows:
  8620.1 (10 Preps): 18 ml (20 ml final vol.)
  8620.2 (50 Preps): 90 ml (100 ml final vol.)
  8620.3 (250 Preps): 162 ml to each bottle (2x 180 ml final vol.)
  Mix thoroughly and keep the bottle always firmly closed!
- Dissolve Proteinase K by addition of distilled H_2O as follows:
  8620.1 (10 Preps): 0.3 ml
  8620.2 (50 Preps): 1.5 ml
  8620.3 (250 Preps): 1.5 ml to each tube
  Mix thoroughly!
- Heat one thermal mixer or water bath to 60 °C.
- Recommended: heat the needed amount of Elution Buffer EB to 60°C. The final elution step with heated Elution Buffer will increase the DNA yield!
  Please note: Centrifugation steps should be carried out at room temperature

4. Workflow

The workflow has been provided on a separate page, in order to use a copy for in-process protocol or filing in your personal lab files.

4.1 Workflow

This workflow describes preparation of gDNA from blood samples of 200 µl. For up-scaling to up to 400 µl see below.

Step (RT = room temperature)	**done**

1. Lysis	
Transfer 200 µl of the blood sample into a 1.5 ml reaction tube. If the sample is less than 200 µl, add 1x PBS up to 200 µl.	
Add 200 µl **Lysis Buffer LSN** and 20 µl Proteinase K solution. Mix vigorously by pulsed vortexing for 10 sec.	
Incubate for 10 mins at 60 °C under constant agitation.*	
Optional: If RNA-free gDNA is required, add 4 µl of RNase A stock solution (100 mg/ml). Vortex shortly and incubate for 5 additional min at RT.	
Centrifuge at full speed for approx. 5 sec. in order to spin down condensed water from the lid.	
Add 350 µl **Binding Buffer BR** to the lysed sample. Mix carefully by pipetting up and down.**	

2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the mix of lysed sample/Binding Buffer BR to the spin column.	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through.***	

3. Column Washing	
Add 400 µl of **Washing Buffer WSO** to the Spin Column	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through***	
Add 600 µl of **Washing Buffer WSH** to the Spin Column	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through***	
Add 600 µl of **Washing Buffer WSH** to the Spin Column	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through***	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 200 µl prewarmed **Elution Buffer EB** to the centre of the membrane****	
Incubate for 2 mins at room temperature	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT to elute DNA	

* We recommend using a shaking platform or thermomixer for continuous agitation of the sample. Alternatively, vortex the sample 3-4 times during incubation. Agitation is vital for a decent recovery rate.
** **Do not vortex** the sample at this point! Vortexing will lead to reduced yield of DNA.
*** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.
*** Elution with lower volumes of Elution Buffer (e.g. 100 µl Elution Buffer only) increases the final concentration of DNA. Repetition of the elution step often increases the yield of gDNA overall, but may reduce the concentration after pooling of the fractions.

4.2 Up-scaling to up to 400 µl blood sample

In case samples of up to 400 µl blood have to be used as source, the following up-scaling for Lysis has to be applied.

Sample vol.	200 µl	250 µl	300 µl	400 µl
Lysis Buffer LSN	200 µl	250 µl	300 µl	400 µl
Proteinase K sol.	20 µl	22 µl	25 µl	30 µl
RNAse A sol.	4µl	4 µl	4 µl	4 µl
Binding Buffer BR	350 µl	440 µl	525 µl	700 µl
Tube size	1.5 ml	1.5 ml	2 ml	2 ml

In most of these cases, the volume resulting from step 1. Lysis is too big for application to the spin column in one step. Hence, Column Loading is performed in two steps:
After lysis has been completed, approx. half of the lysed sample / Binding Buffer BR mix is added to the centre of the spin column membrane. After centrifugation, the second half of the mix is filtered through the membrane.

Step **3. Column Washing**, and step **4. Elution**, are performed without any changes in the amounts given for isolation from 200 µl blood.

Up-scaling protocol for your specific data

Step (RT = room temperature)	**done**

1. Lysis	
Transfer ..... µl of the blood sample into a 1.5 ml reaction tube.	
Add ..... µl **Lysis Buffer LSN** and ..... µl Proteinase K solution. Mix vigorously by pulsed vortexing for 10 sec.	
Incubate for 10 mins at 60 °C under constant agitation.*	
Optional: If RNA-free gDNA is required, add 4 µl of RNase A stock solution (100 mg/ml). Vortex shortly and incubate for 5 additional min at RT.	
Centrifuge at full speed for approx. 5 sec.	
Add ..... µl **Binding Buffer BR** to the lysed sample. Mix carefully by pipetting up and down.**	

2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply approx. half of the mix of lysed sample/Binding Buffer BR to the spin column.	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through.***	
Apply residual mix of lysed sample/Binding Buffer BR to the spin column.	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through.***	

3. Column Washing	
Add 400 µl of **Washing Buffer WSO** to the Spin Column	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through***	
Add 600 µl of **Washing Buffer WSH** to the Spin Column	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through***	
Add 600 µl of **Washing Buffer WSH** to the Spin Column	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through***	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 200 µl prewarmed **Elution Buffer EB** to the centre of the membrane****	
Incubate for 2 mins at room temperature	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT to elute DNA	

* We recommend using a shaking platform or thermomixer for continuous agitation of the sample. Alternatively, vortex the sample 3-4 times during incubation. Agitation is vital for a decent recovery rate.

** **Do not vortex** the sample at this point! Vortexing will lead to reduced yield of DNA.

*** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.

*** Elution with lower volumes of Elution Buffer (e.g. 100 µl Elution Buffer only) increases the final concentration of DNA. Repetition of the elution step often increases the yield of gDNA overall, but may reduce the concentration after pooling of the fractions.

5. Trouble Shooting

Problem / probable cause	Comments and suggestions
Clogged spin filter	
Insufficient lysis and/or too much starting material	Increase lysis time.
	Increase centrifugation speed.
	After lysis centrifuge the lysate to pellet unlysed material.
	Check storage conditions and usage of Proteinase K. Optionally replace Proteinase K by a fresh lot.
	Reduce amount of starting material.
Low recovery	
Insufficient lysis	See above
Insufficient mixing with Binding Buffer BR	Mix sample very well with Binding Buffer BR by pipetting prior to transfer of the sample onto the Spin Filter
Low amount of recovered gDNA.	Add the Elution Buffer directly onto the centre of the Spin Column.
	Prolong the incubation time with Elution Buffer.
	Increase volume of Elution Buffer used or repeat elution step.
Low concentration of eluted gDNA	Reduce the volume of Elution Buffer used. Optionally: prolong elution / incubation time.
Degraded or sheared DNA	
Incorrect storage of starting material.	Make sure that the starting material is frozen immediately in liquid N_2 or in minimum at -20 °C, and is stored continuously at -80 °C!
	Avoid repeated freezing and thawing of the starting material.
Old material	Use fresh material. Check and improve storage conditions.
RNA contamination	
Unsufficient RNAse digestion during lysis	Add RNAse A after lysis.
	Check storage conditions and usage of RNAse A. Optionally replace RNAse A by a fresh lot.

***Your notes*:**

Ordering information:

(for detailed kit content see Table under 1.)

Product number	Product	Content	Amount
8620.1	**Roti®-Prep Blood Genomic DNA MINI**	1 Test-Kit	10 Preps
8620.2	**Roti®-Prep Blood Genomic DNA MINI**	1 Kit	50 Preps
8620.3	**Roti®-Prep Blood Genomic DNA MINI**	1 Maxi-Kit	250 Preps

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